

In the claims:

1. **(Currently amended)** A method for preparing liposomes containing at least one biologically active substance encapsulated therein under mild conditions, said method comprising the following steps:

(A) providing liposomes, wherein the liposomes are prepared by a method other than the instant method;

(B) mixing the product of step (A) with aqueous medium U and a water-miscible organic solvent to form a gel or a liquid containing gel particles; and thereafter

(C) (a) mixing the gel or liquid containing gel particles with aqueous medium V to directly form the liposomes containing the at least one biologically active substance encapsulated therein,

(b) [(i)] mixing the gel or liquid containing gel particles with aqueous medium V and then mixing with aqueous medium W to directly form the liposomes containing the at least one biologically active substance encapsulated therein, to form a curd or curdy substance; and

—————(ii) ~~mixing the curd or curdy substance with aqueous medium W to directly form the liposomes containing the at least one biologically active substance encapsulated therein; or~~

(c) [(i)] cooling the gel or liquid containing gel particles to form a waxy substance; and then

[(ii)] mixing the waxy substance with aqueous medium W to directly form the liposomes containing the at least one biologically active substance encapsulated therein;

wherein the at least one biologically active substance is added in step (A), step (B) and/or step (C), and wherein aqueous media U, V and W are the same or different.

2. **(Canceled).**

3. **(Currently amended)** The method of claim 1 [[2]], wherein the liposomes containing the at least one biologically active substance encapsulated therein of step (C) are washed with an aqueous medium by centrifugation, gel filtration or dialysis.

4. **(Currently amended)** The method of claim 1 [[2]], wherein the organic solvent is selected from the group consisting of acetaldehyde, acetone, acetonitrile, allyl alcohol, allylamine, 2-amino-1-butanol, 1-aminoethanol, 2-aminoethanol, 2-amino-2-ethyl-1,3-propanediol, 2-amino-2-methyl-1-propanol, 3-aminopentane, N-(3-aminopropyl)morpholine, benzylamine, bis(2-ethoxyethyl) ether, bis(2-hydroxyethyl) ether, bis(2-hydroxypropyl) ether, bis(2-methoxyethyl) ether, 2-bromoethanol, meso-2,3-butanediol, 2-(2-butoxyethoxy)-ethanol, butylamine, secbutylamine, tert-butylamine, 4-butyrolactone, 2-chloroethanol, 1-chloro-2-propanol, 2-cyanoethanol, 3-cyanopyridine, cyclohexylamine, diethylamine, diethylenetriamine, N,N-diethylformamide, 1,2-dihydroxy-4-methylbenzene, N,N-dimethylacetamide, N,N-dimethylformamide, 2,6-dimethylmorpholine, 1,4-dioxane, 1,3-dioxolane, dipentaerythritol, ethanol, 2,3-epoxy-1-propanol, 2-ethoxyethanol, 2-(2-ethoxyethoxy)-ethanol, 2-(2-ethoxyethoxy)-ethyl acetate, ethylamine, 2-(ethylamino)ethanol, ethylene glycol, ethylene oxide, ethylenimine, ethyl(-)lactate, N-ethylmorpholine, ethyl-2-pyridine-carboxylate, formamide, furfuryl alcohol, furfurylamine, glutaric dialdehyde, glycerol, hexamethylphosphoramide, 2,5-hexanedione, hydroxyacetone, 2-hydroxyethyl-hydrazine, N-(2-hydroxyethyl)morpholine, 4-hydroxy-4-methyl-2-pentanone, 5-hydroxy-2-pentanone, 2-hydroxypropionitrile, 3-hydroxypropionitrile, 1-(2-hydroxy-1-propoxy)-

2-propanol, isobutylamine, isopropylamine, 2-isopropylamino-ethanol, 2-mercaptoethanol, methanol, 3-methoxy-1-butanol, 2-methoxyethanol, 2-(2-methoxyethoxy)-ethanol, 1-methoxy-2-propanol, 2-(methylamino)-ethanol, 1-methylbutylamine, methylhydrazine, methyl hydroperoxide, 2-methylpyridine, 3-methylpyridine, 4-methylpyridine, N-methylpyrrolidine, N-methyl-2-pyrrolidinone, morpholine, nicotine, piperidine, 1,2-propanediol, 1,3-propanediol, 1-propanol, 2-propanol, propylamine, propyleneimine, 2-propyn-1-ol, pyridine, pyrimidine, pyrrolidine, 2-pyrrolidinone and quinoxaline.

5. **(Original)** The method of claim 4, wherein the organic solvent is acetonitrile, acetone or a C₁-C₃ alcohol.

6. **(Original)** The method of claim 5, wherein the organic solvent is methanol, ethanol, 1-propanol, 2-propanol, ethylene glycol or propylene glycol.

7. **(Original)** The method of claim 6, wherein the organic solvent is ethanol, 1-propanol or 2-propanol.

8. **(Original)** The method of claim 7, wherein the organic solvent is ethanol.

9. **(Original)** The method of claim 4, wherein the organic solvent is acetone.

10. **(Currently amended)** The method of claim 1[[2]], wherein aqueous medium U, aqueous medium V and/or aqueous medium W is an aqueous buffer.

11. **(Currently amended)** The method of claim 1[[2]], wherein the gel or liquid and aqueous medium V are mixed in step (C) by adding aqueous medium V to the gel or the liquid containing gel particles.

12. **(Currently amended)** The method of claim 1 [[2]], wherein the gel or the liquid containing gel particles and aqueous medium V are mixed in step (C) by adding or infusing the gel or liquid into aqueous medium V.

13. **(Currently amended)** The method of claim 1 [[2]], wherein the at least one biologically active substance is a nucleic acid, pharmaceutical agent, diagnostic agent, protein, peptide, antigen, cytochrome C, transcription factor, cytokine or hapten.

14. **(Original)** The method of claim 13, wherein the at least one biologically active substance is a plasmid DNA.

15. **(Original)** The method of claim 14, wherein the plasmid DNA is up to about 20 kb in size.

16. **(Original)** The method of claim 15, wherein the plasmid DNA is of from about 0.5 kb to about 20 kb in size.

17. **(Original)** The method of claim 16, wherein the plasmid DNA is of about 1 kb to about 15 kb in size.

18. **(Original)** The method of claim 17, wherein the plasmid DNA is of about 2 kb to about 10 kb in size.

19. **(Original)** The method of claim 18, wherein the plasmid DNA is of about 3 kb to about 7 kb in size.

20. **(Currently amended)** The method of claim 1 [[2]], wherein the at least one biologically active substance is selected from the group consisting of proteins and antigens structurally sensitive to dehydration.

21. **(Original)** The method of claim 20, wherein the proteins and antigens structurally sensitive to dehydration are tetanus toxoids.

22. **(Currently amended)** The method of claim 1 [2], wherein the at least one biologically active substance is at least one pharmaceutical agent selected from the group consisting of anti-neoplastic agents, anti-microbial agents, anti-viral agents, antihypertensive agents, anti-inflammatory agents, bronchodilators, local anesthetics and immunosuppressants.

23. **(Original)** The method of claim 22, wherein the at least one pharmaceutical agent is selected from the group consisting of anti-bacterial agents and anti-fungal agents.

24. **(Original)** The method of claim 22, wherein the at least one pharmaceutical agent is selected from the group consisting of anti-fungal agents and anti-neoplastic agents.

25. **(Currently amended)** The method of claim 1 [2], wherein the at least one biologically active substance is a bioreactive lipid.

26. **(Currently amended)** The method of claim 1 [2], wherein the at least one biologically active substance is an antibody, enzyme or cytokine.

27. **(Currently amended)** The method of claim 1 [2], wherein the at least one biologically active substance is an RNA.

28. **(Currently amended)** The method of claim 1 [2], wherein the at least one biologically active substance is an oligonucleotide.

29. **(Original)** The method of claim 28, wherein the at least one biologically active substance is an oligonucleotide of about 5 to about 500 bases in size.

30. **(Currently amended)** The method of claim 1 [2], wherein the liposome of step (A) further comprises a sterol.

31. **(Original)** The method of claim 30, wherein the sterol is cholesterol.

32.-62 **(Canceled)**.

63. **(Currently amended)** The method of claim 1 [[2]], wherein the liposomes of step (A) comprise at least one fusogenic lipid.

64. **(Original)** The method of claim 63, wherein the at least one fusogenic lipid is selected from the group consisting of N-acyl phosphatidylethanolamine.

65. **(Original)** The method of claim 64, wherein the at least one fusogenic lipid is selected from the group consisting of N-decanoyl phosphatidylethanolamine, N-dodecanoyl phosphatidylethanolamine and N-tetradecanoyl phosphatidylethanolamine.

66. **(Original)** The method of claim 65, wherein the at least one fusogenic lipid is selected from the group consisting of N-dodecanoyl phosphatidylethanolamine.

67-74.- **(Canceled)**.

75. **(Currently amended)** The method of claim 1 [[2]], wherein aqueous medium V is mixed in increments with the gel or the liquid containing gel particles in step (C), wherein the increments are up to about 100% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

76. **(Original)** The method of claim 75, wherein the increments are up to about 80 of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

77. **(Original)** The method of claim 76, wherein the increments are up to about 60% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

78. **(Original)** The method of claim 77, wherein the increments are up to about 40 of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

79. **(Original)** The method of claim 78, wherein the increments are up to about 20 of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

80. **(Original)** The method of claim 79, wherein the increments are up to about 10 of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

81. **(Original)** The method of claim 80, wherein the increments are up to about 5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

82. **(Original)** The method of claim 81, wherein the increments are up to about 1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

83. **(Original)** The method of claim 82, wherein the increments are up to about 0.5 of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

84. **(Original)** The method of claim 83, wherein the increments are up to about 0.1 of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

85. **(Original)** The method of claim 80, wherein the increments are from about 0.001% to about 10% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

86. **(Original)** The method of claim 85, wherein the increments are from about 0.001% to about 5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

87. **(Original)** The method of claim 86, wherein the increments are from about 0.001% to about 1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium V.

88.-115. **(Canceled).**

116. **(Currently amended)** The method of claim 1 ~~[[2]]~~, wherein the liposomes provided in step (A)~~(a), (A)(b), (A)(c), (A)(d) or (A)(f) comprise at least one charged lipid, the liposomes in step (A)(e)~~ are formed in the presence of at least one charged lipid and the at least one biologically active substance, or at least one charged lipid is added in step (B), wherein the at least one charged lipid is a lipid ~~[[c]]~~having a net negative or positive charge.

117. **(Original)** The method of claim 116, wherein the at least one charged lipid is selected from the group consisting of N-acyl phosphatidylethanolamine, phosphatidylserine, phosphatidylnositol, phosphatidylglycerol, diphosphatidylglycerol and phosphatidic acid.

118. **(Original)** The method of claim 116, wherein the at least one charged lipid is liposome forming.

119-124. **(Canceled).**

125. **(Currently amended)** The method of claim 1 [2], wherein the amount of lipid in the gel or the liquid containing gel particles of step (B) ranges from 1% by weight of the gel or the liquid containing gel particles to the hydration limit of the lipid in water, wherein the "hydration limit" is the maximum amount of lipid in a given amount of water that would keep the lipid in a liposomal state.

126. **(Currently amended)** The method of claim 1 [2], wherein the amount of lipid in the gel or the liquid containing gel particles of step (B) ranges from about 5% to about 95% by weight of the gel or the liquid containing gel particles.

127. **(Original)** The method of claim 126, wherein said amount of lipid ranges from about 10% to about 95% by weight of the gel or the liquid containing gel particles.

128. **(Original)** The method of claim 127, wherein said amount of lipid ranges from about 15% to about 95% by weight of the gel or the liquid containing gel particles.

129. **(Original)** The method of claim 128, wherein said amount of lipid ranges from about 20% to about 95% by weight of the gel or the liquid containing gel particles.

130. **(Original)** The method of claim 129, wherein said amount of lipid ranges from about 30% to about 95% by weight of the gel or the liquid containing gel particles.

131. **(Original)** The method of claim 130, wherein said amount of lipid ranges from about 40% to about 95% by weight of the gel or the liquid containing gel particles.

132. **(Original)** The method of claim 131, wherein said amount of lipid ranges from about 50% to about 95% by weight of the gel or the liquid containing gel particles.

133. **(Original)** The method of claim 132, wherein said amount of lipid ranges from about 60% to about 95% by weight of the gel or the liquid containing gel particles.

134. **(Original)** The method of claim 133, wherein said amount of lipid ranges from about 70% to about 95% by weight of the gel or the liquid containing gel particles.

135-154. **(Canceled)**.